BIPHASIC RESPONSE OF SALIVARY PERFORMANCE IN MICE FOLLOWING NICOTINE TARTRATE*

by

C. G. Van Dongen
Bio-Research Institute
Cambridge, Mass. 02141

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Mr. Chairman, Members of the Society:

We reported previously on a new method for the quantitative measurement of salivation in mice. The method is based on a sensitive physiological response, is non-traumatic to the animal, does not require anesthesia, provides information on immediate and after-effects to applied treatments, measures quantitative rather than qualitative, allows for simultaneous measurements on large numbers of mice, and is thus suitable for adequate statistical analysis of the experimental data.

Since this methodology possesses certain intrinsic advantages, it is important to make a detailed characterization of the salivary response in this system and to compare wherever possible the obtained results with those published in other species and differing methodologies.

In this respect, we found that various physiological factors such as sex and strain do affect the salivary performance. Exposure to tobacco smokes inhibits salivation. The degree and duration are dependent on the dose. The known "salivation inhibitory drug " atropine-sulfate was found to inhibit the salivation in a dose-related fashion, whereas the known "salivation stimulatory drug" metacholine shows a dramatic and dose-related initial salivary stimulation followed by an equally dramatic salivary inhibition. The dose-response relationship with the two drugs were so striking and reproducible that it was suggested to use the salivary measurement in mice as a biological assay for cholinergic and anticholinergic drug substances.

In an effort to broaden the experimental data base of the mouse salivation method for improved standardization and methodological comparisons, and to aid in the understanding of the control of salivary secretion in the mouse, we have now selected to study the ganglionic blocking agent nicotine. According to the literature, nicotine is known to have a biphasic action on salivation, namely that of an initial stimulation followed by an inhibition. The highest salivary stimulations can be expected with the lowest nicotine dosages. Although this information is generally true, it is well known that extreme variability in response exists from animal to animal, which makes the biphasic salivary response often unreproducible. The underlying physiological causes for this variability are not well understood.

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The specific objectives of this study are: (1) to confirm or disprove a biphasic response of nicotine on the salivary performance of mice; (2) to determine the dose-dependence of the response; (3) to examine the validity of the above for a "genetically high" and a "genetically low" salivating strain of mice.

The two strains of mice selected for this study were the "high-salivating" C57BL and the "low-salivating" AKR strain, both obtained from the Jackson Laboratories in Bar Harbor, Maine. Of each strain, a large herd of male mice was available.

The first slide illustrates the measurement of salivary performances in various strains of mice: high-salivating C57BL mice; low-salivating AKR mice.

The next slide shows the experimental layout of a single day's experiment with either the C57BL or AKR mice. Salivary performances of 36 mice were determined four times with a one-hour interval between measurements. The first and second measurements served to establish the control salivary performance of individual mice during that particular day. The mice were then rankordered according to salivary performance and systematically assigned to any one of six treatment groups. The various treatments were applied, immediately prior to the third measurement. The fourth measurement was performed to examine any after-effects to treatments. Nicotine tartrate was injected subcutaneously in 0.1 ml of water.

It is noteworthy that any treatment can be compared validly with two types of controls, namely first with "no-injection," and "injection of water," but also second with their own salivary performances during the first and second measurements, as earlier results had shown that non-significant differences in salivation exist during the day.

A single day's experiment was repeated 4 times for each strain. Therefore, the entire study was done on approximately 288 mice and provided 1152 salivation measurements. The salivary responses to nicotine were examined qualitatively and quantitatively.

We will first present some qualitative data.

The next slide shows the frequencies of an increase in salivary performance by individual mice immediately following the applied treatment. More than 50% would indicate an overall increase, and less than 50% a decrease. As you see, the overall immediate response to

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nicotine seems that of slight salivary stimulation. A closer look at the data reveals that increases are more prevalent with low-salivating than with high-salivating animals. This finding holds true among strains and among individual animals within each strain.

The next slide shows the frequencies of salivary increases one-hour after injection. The predominant effect now is that of salivary inhibition, with the possible exception of low-salivating individuals of the C57BL strain.

The qualitative data thus indicate that nicotine increases salivary performance initially, followed by a salivary inhibition, and that this effect shows up most clearly with the low-salivating individuals of each strain.

We will now turn our attention to quantitative observations.

The next slide shows the effect of nicotine tartrate on the salivary performance of the low-salivating AKR mice, which have a base-salivation of approximately 25 mm of boundary displacement. Note first of all, that the control and water injections are approximately stable over time. The injection with nicotine shows an immediate stimulatory or "no effect," the highest stimulation occurring with the lowest dose. The after-effect is that of a salivary inhibition, the degree of which is positive related to the dose.

The next slide shows the salivary performances of high- or low-salivating individuals of the AKR strain in response to one of 4 doses of nicotine tartrate. Note that in low-salivating individuals, salivation is initially increased at all dose levels and is followed by slight decreases when compared with the salivary performance during the second measurement. With the high-salivating AKR's, only the lowest dose of nicotine showed a dramatic initial stimulatory effect, a slight stimulation was noticed with 0.30 mg, whereas 0.10 and 1.00 mg did not show any effect. The after-effect was clearly that of a salivary inhibition which seems to be dose related.

The next slide shows the effect of various doses of nicotine on the salivary performance of the high-salivating C57BL strain, whose mean base-salivation level is approximately 55 mm of boundary displacement. Here again, the two controls are reasonably stable over time. Note further that the most dramatic initial salivary stimulation occurs with the lowest nicotine dose, whereas the initial salivary decreases are obtained with higher nicotine doses. The after-

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effect to nicotine is again a salivary inhibition.

The next slide shows the effect of various doses of nicotine on the salivary performances of low- and high-salivating individuals of this high-salivating strain of mice. In the low-salivators, the immediate response to nicotine was in 3 of 4 dosages that of a stimulation, with the most dramatic one showing up at the lowest nicotine dose. The after-effect was that of slight inhibition. With high-salivators, on the contrary, all nicotine dosages were inhibitory to salivation, both initially and one-hour after injection.

The next slide shows a comparison of the salivary performances of AKR vs. C57BL mice in response to various doses of nicotine tartrate. At the lowest nicotine dose, both strains show a clearcut initial stimulation. With the three higher nicotine doses, slight stimulations can be observed with the AKR's and salivary inhibitions with the C57BL's.

The obtained data with mice support results published in the literature with different species, with respect to the presence of a biphasic response on salivation by nicotine and with respect to its dose-dependence. Our results show that this phenomenon holds in principle true in two different strains of mice, but that quantitatively, the base salivation level of mice is of critical importance. Physiologically, our findings can be explained as follows: salivary performance consists of two components, namely spontaneous salivation and induced salivation. Induced salivation is under direct neural control, both through the sympathetic and parasympathetic nerve systems. Nicotine stimulates the ganglion cells at low concentration. At higher concentration, it blocks them with as a result a prolonged salivary inhibition.

In Summary:

A new salivation method in mice was used to study the effect of various doses of subcutaneously injected nicotine tartrate on the salivary performance of high- and low-salivating individuals from two strains of mice. Nicotine exerts a biphasic response on salivation in mice. To notice these effects, the salivary level of the mice and the dose of nicotine employed are major factors. The strain of mice per se does not seem to exert any influence. The results are in agreement with information to this effect in other species and can be explained with the ganglionic action of nicotine.

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EXPERIMENTAL DESIGN FOR A SINGLE DAY'S EXPERIMENT, STUDYING THE EFFECT OF VARIOUS DOSES OF NICOTINE TARTRATE ON THE SALIVARY PERFORMANCE OF MALE C57BL/J MICE. (INJECTION SUBCUTANEOUSLY AND IMMEDIATELY PRIOR TO THE THIRD MEASUREMENT)

Treatment	No. of animals	Measurement			
		1	2	3	4
No injection	6				
Injection with H_2O	6				
0.03 mg nic. tart.	6				
0.10 mg nic. tart.	6				
0.30 mg nic. tart.	6				
1.00 mg nic. tart.	6				

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Number of Mice That Show an Increased Salivary Performance Immediately after a Subcutaneous Injection with Nicotine Tartrate (When Compared to the Second Salivation Measurement and Expressed as Percentage)

Strain	Salivation Level		Overall
	"Low"	"High"	
AKR/J	70	56	63
C57BL/6J	63	31	47
Combined	66	43	54

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Number of Mice That Show an Increased Salivary Performance One Hour after a Subcutaneous Injection with Nicotine Tartrate (When Compared to the Second Salivation Measurement and Expressed as Percentage)

Strain	Salivation Level		Overall
	"Low"	"High"	
AKR/J	38	29	33
C57BL/6J	62	23	42
Combined	50	25	38

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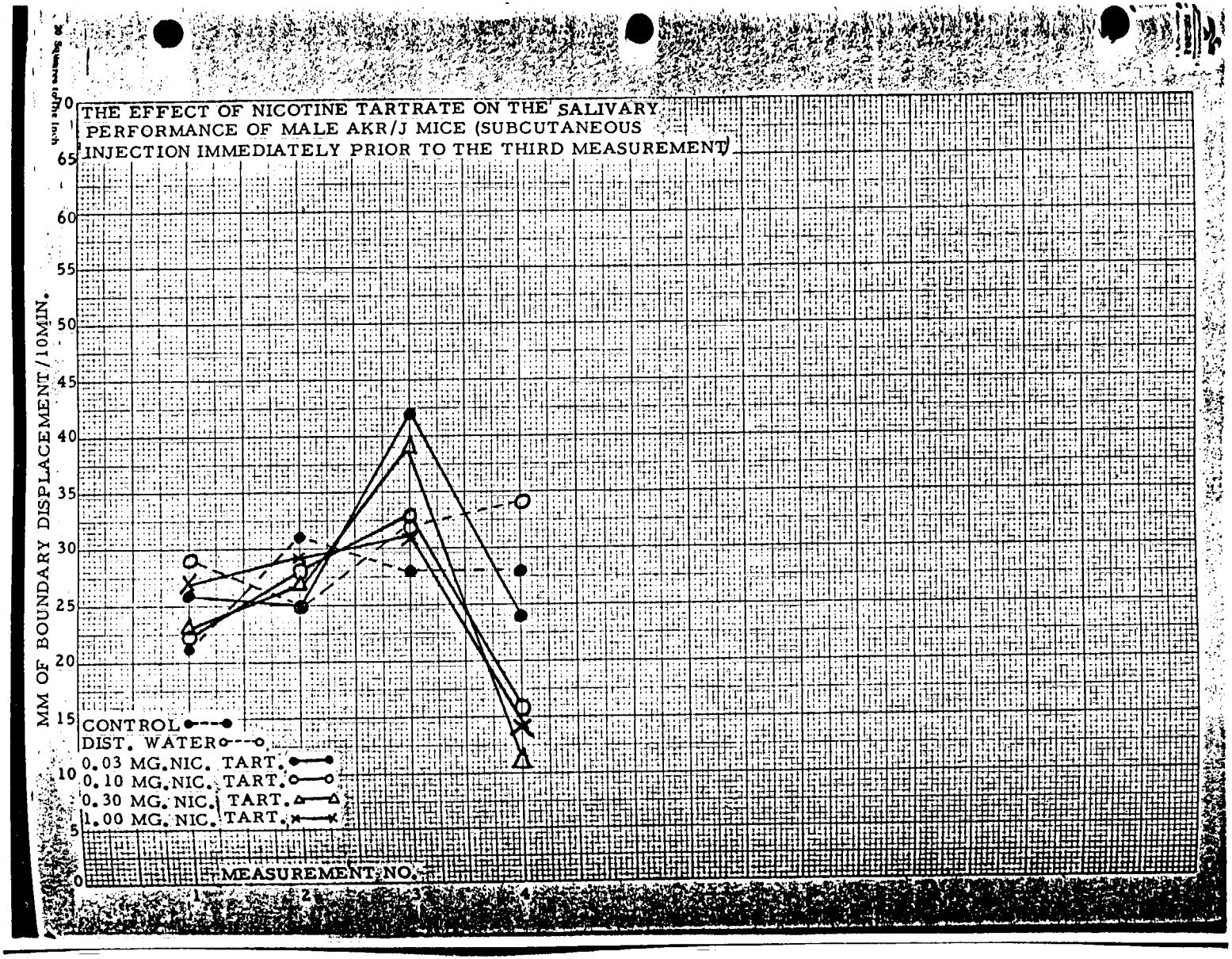

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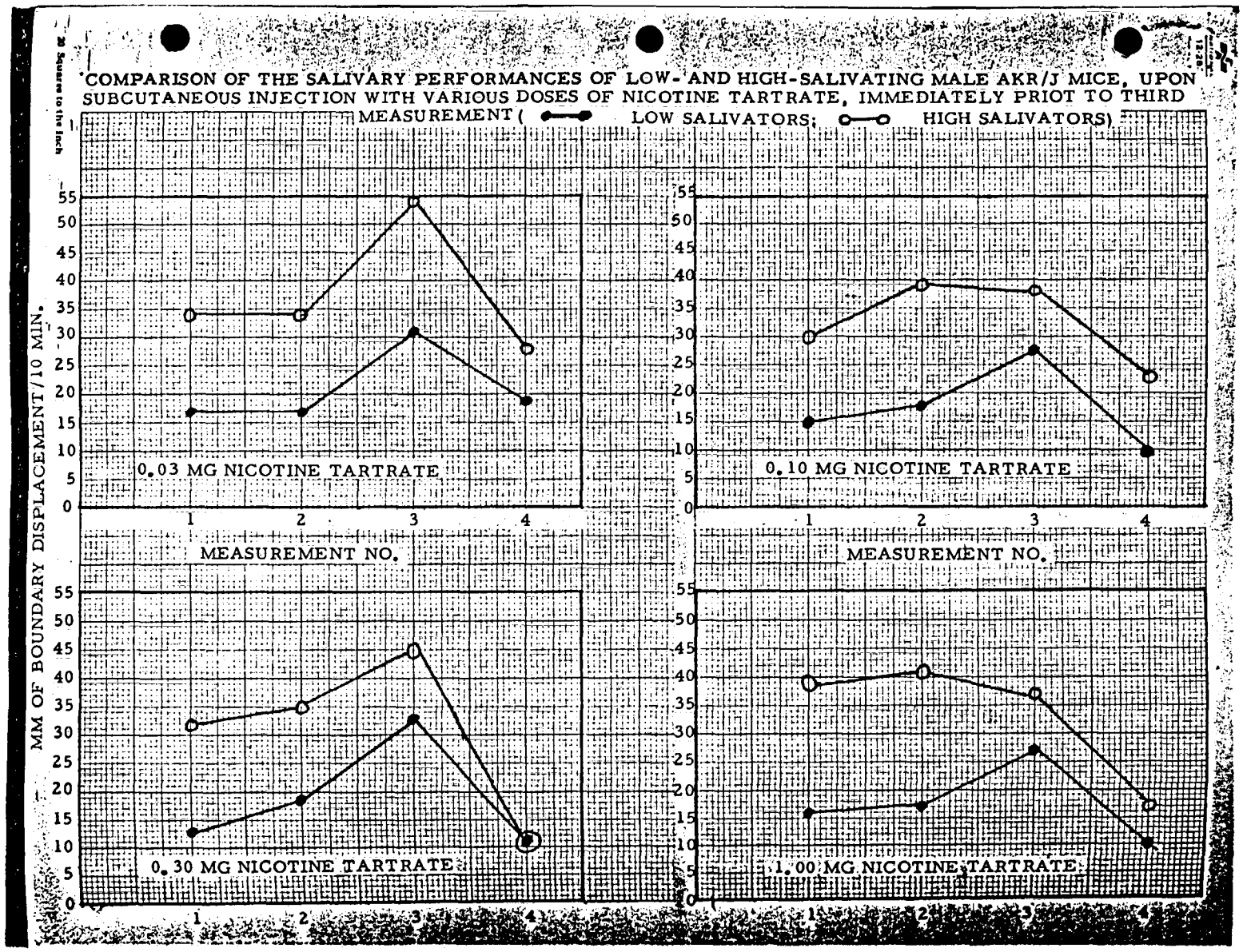

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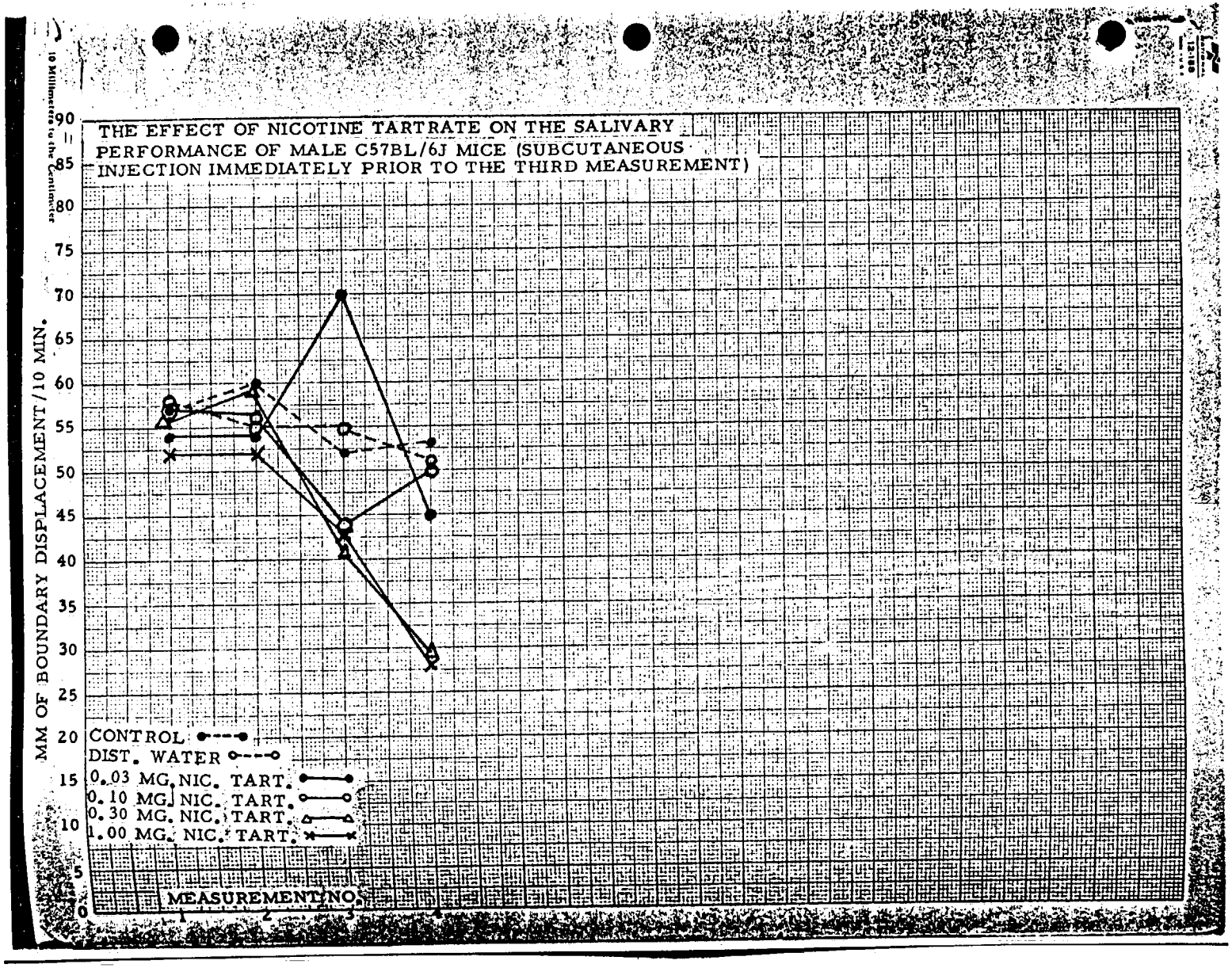

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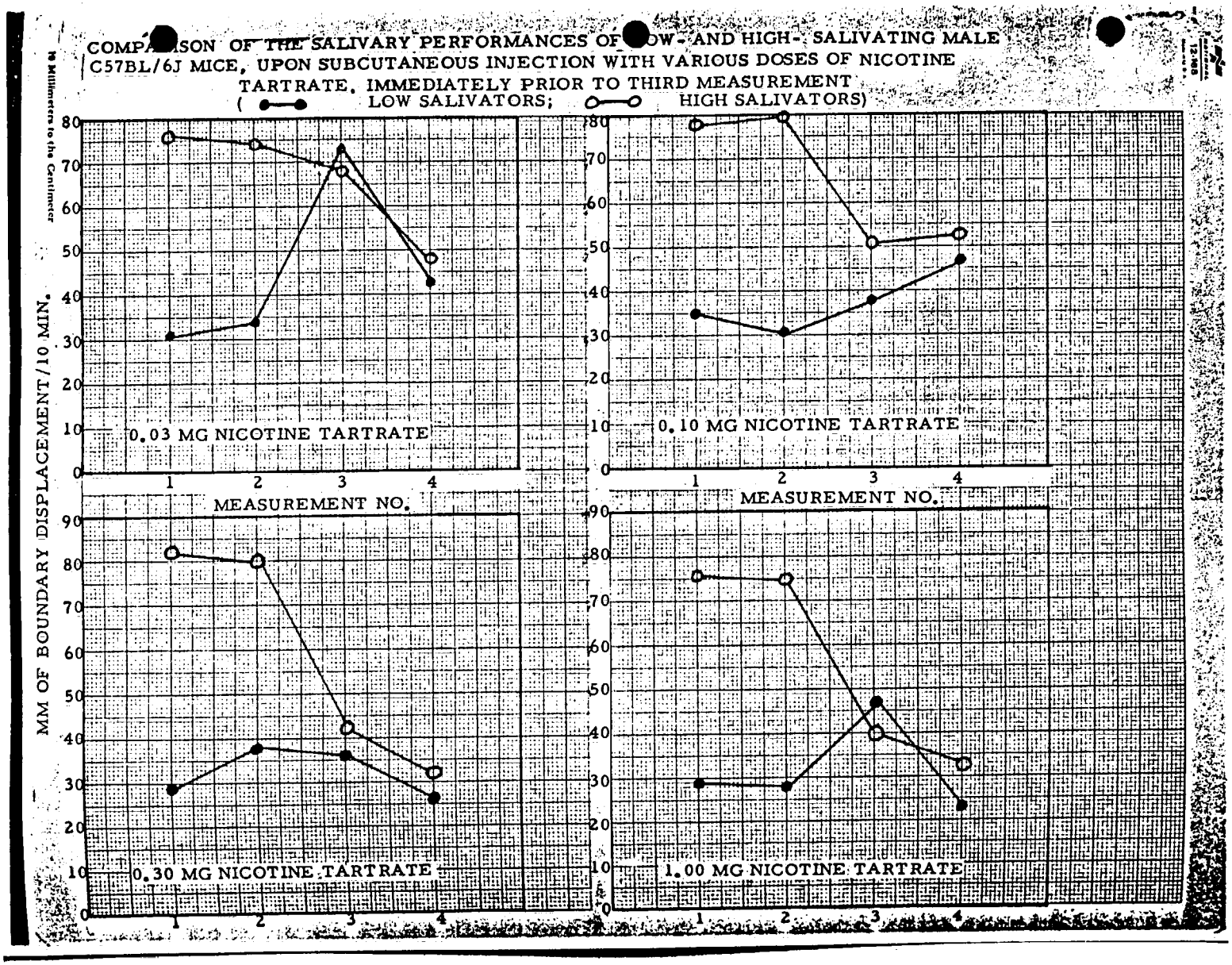

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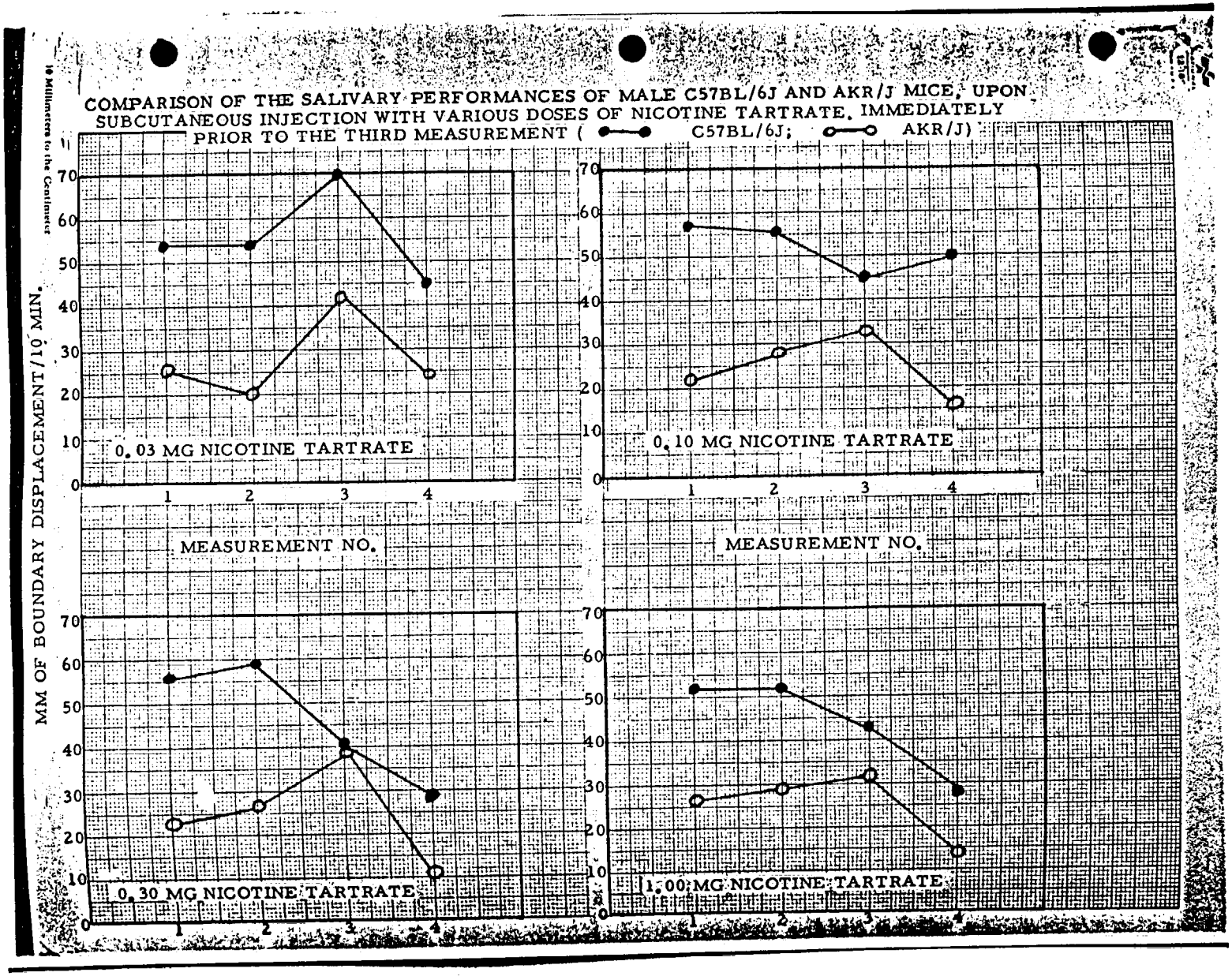

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